Short Communication

PHASE SOLUBILITY ANALYSIS: AN EVALUATION OF THE TECHNIQUE

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The increasing prominence given to the need for reference standards has prompted an investigation of possible assay methods for assessing the purity of such substances. The advantages claimed for phase solubility analysis made this an obvious choice for examination. Our investigations arose in part from work undertaken by one of us (D.C.G.) as a member of the Synthetic Drugs Committee of the British Pharmacopoeia Commission.

Parsons, Genarro and Osol (1961) have used phase solubility analysis in assaying adrenocortical steroid reference standards. This method has been fully described by Mader (1954) but as far as we are aware has received little attention in this country. It is based on the principles of Gibbs' phase rule and involves equilibrating varying weights of solid with a fixed weight of solvent in sealed ampoules at constant temperature and pressure. When equilibrium has been established the weights of solute per gram of solvent (Solution Concentration) are determined and plotted against the weights of solid originally taken per gram of solvent (System Concentration). A phase solubility curve is obtained and the percentage total impurity is calculated from the slope of the curve immediately following the first turning point.

For a pure compound the slope will be zero whilst for a compound containing one impurity the slope (expressed as a percentage) gives the impurity. For a compound containing several impurities several turning points, each corresponding to an impurity, may be obtained. The total impurity is calculated from the slope of the curve following the first turning point whilst the individual impurities are obtained from the difference in the slopes of the curves on either side of the succeeding turning points.

Table I lists the results obtained by this and other methods of assay. It shows that, when applied to the analysis of mecamylamine hydrochloride, the method outlined in the experimental section gave results reproducible to within $\pm 0{\cdot}3$ per cent.

At this stage in our investigation it is our opinion that the sensitivity and selectivity of the technique outweigh the disadvantages of lengthy analysis time. Furthermore, we feel that this technique may be of further value not only in assessing the purity of possible reference standards, but in the detection of closely-related impurities in new synthetic drugs.

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EXPERIMENTAL

Various techniques have been described for this type of assay (Tarpley and Yudis, 1953; Outch, 1961; Parsons and others, 1961). The reasons underlying our choice of technique are outlined below.

(i) Neutral glass ampoules were used since the possibility of an impurity being formed during equilibration could not be discounted with soda glass. Ampoules were rinsed once with 5 per cent acetic acid, 6 times with distilled water, then dried at 105°.

(ii) Solvents or mixed solvents in which the solubility of the compound being determined is between 0·4 and 2·5 per cent, as recommended, have been found to be satisfactory (Mader, 1954). The boiling-points should ideally lie between 60–100°. It was found unnecessary to chill the ampoules during the sealing operation.

TABLE I

Compound	Solvent system and solubility at 25°	Phase solubility assay		Tetrazolium assay per cent compound	Ultraviolet* assay per cent compound
		per cent impurity	per cent compound		
ydrocortisone B.P.	14·2 mg./g. in methanol	3·3	96·7	99·4 100·0	100·4
Hydrocortisone B.P.	14·2 mg./g. in methanol	1·7	98·3	99·4 99·0	99·3
Prednisolone†	5·96 mg./g. in benzene-methanol (95 + 5)	12·5	87·5	92·5 93·3	99·0
				Chloride assay per cent compound	
Mecamylamine hydrochloride	19·7 mg./g. in isopropanol				
Batch 1		4·6; 4·7	95·4; 95·3	100·7; 100·6; 100·7	
Batch 2 equilibrated 2 days		1·8; 1·7 1·9; 2·0; 1·8	98·2; 98·3 98·2; 98·0; 98·1	99·9; 100·1; 100·3	
Batch 2 equilibrated 10 days		2·1; 1·6	97·9; 98·4		

* Official method of the British Pharmacopoeia, 1958.
† Process sample before purification.

(iii) The period of equilibration varies from system to system. Four weeks has been used for corticosteroids, whilst 2 days has been found sufficient for mecamylamine hydrochloride.

(iv) Having separated the liquid from the solid phase, the solution concentration is determined gravimetrically after evaporation of the solvent. The method of evaporation is governed by the thermal stability of the compounds being examined. Evaporation on a steam-bath whilst a gentle stream of oxygen-free nitrogen is blown across the surface of the solvent has been found to be satisfactory for hydrocortisone in methanol, prednisolone in benzene-methanol and mecamylamine hydrochloride in isopropanol.

(v) Slopes of the curves have been calculated statistically to obtain the line of best fit. Small variations in the technique are thus averaged out and an accurate measure of the impurity obtained.

All solvents were of analytical reagent grade. Cotton Wool B.P.C. was washed with methanol and dried at 100°. Substances under investigation were all finely ground and dried *in vacuo* at room temperature over phosphorus pentoxide.

Neutral glass ampoules (20 *ml.*). Rinsed once with 5 per cent acetic acid followed by six water washings and dried at 105°.

Procedure. Transfer the required amounts of substance to eight clean, dry, tared glass ampoules, each of 20 ml. capacity, by means of a long-stemmed funnel, taking care to avoid contaminating the neck of the ampoule. Re-weigh each ampoule, add solvent (15 ml.) from a glass syringe and flame seal the ampoules; cool and re-weigh. The concentration in one of the ampoules should lie below the saturation point, whilst the system concentration in the remaining seven should be varied from just above the saturation point to five times this concentration. (Solvent systems and solubilities of the substances examined are given in Table I.)

Equilibrate the ampoules by lengthwise rotation (50 r.p.m.) in a water-bath at 25·0 (±0·1°) for the specified time. Support the ampoules vertically in the bath for a further 24 hr. to allow the solid phase to settle.

Remove a sample of solution (10 ml.) whilst the ampoules are still in the bath; use a glass syringe fitted with a 3½ in. × 17 G hypodermic needle. The needle should be fitted with a stop, to prevent the solid phase being disturbed, and the attachment end should contain a small plug of cotton wool to act as a filter. Remove the needle and immediately transfer the solution to a dried, tared (to the nearest 0·01 mg.) weighing bottle (25 ml.) fitted with a ground-glass stopper, and re-weigh. Evaporate the solution to a volume of 1 ml. as in (iv) above, remove the last traces of solvent at room temperature with oxygen-free nitrogen. Dry to constant weight in a vacuum desiccator over phosphorus pentoxide at a pressure not exceeding 5 mm. mercury.

A plot of the system concentration (X mg./g. of solvent) versus the solution concentration (Y mg./g. of solvent) should give a straight line if equilibrium has been reached.

$$\text{per cent total impurity} = \frac{100 \times \Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}$$

where N = number of ordinates of the first slope.

References

Mader, W. (1954). *Organic Analysis*, Vol. II, p. 253, New York, Interscience Publishers.
Parsons, W. H., Genarro, A. R. and Osol, A. (1961). *Amer. J. Pharm.*, **133**, 351–359.
Outch, W. T. (1961). *Aust. J. Pharmacy*, **42**, 1227–1283.
Tarpley, W. and Yudis, M. (1953). *Analyt. Chem.*, **25**, 121–127.

Discussion

The paper was presented by Mr. Johnson. The following points were made in the discussion.

The time of equilibration might be influenced by a number of factors. It has been suggested that solid particles in contact with a liquid become surrounded by a thin film of solution and that the solid then diffuses through this film into the bulk of the liquid; the rate of this diffusion depends on the nature of the solid, the temperature, and the viscosity of the surrounding film. Particle size might also play a part since a finely divided sample sometimes has a greater solubility than one less finely divided. The extent to which supersaturation occurs would affect the time of equilibration and, in the case of the steroids, polymorphism may play a part since the same substance in the two different crystalline forms would behave as two components. The phase solubility method would fail if two components were present in the same ratio as their solubilities; this could be overcome by choosing a different solvent system or changing the temperature of equilibration. Other causes of failure were a non-ideal behaviour of the solution (avoided by working with suitably dilute solutions), the formation of solid solutions (frequently overcome by using a different solvent system) or some variation in the chemical nature of the solute due to reaction with the solvent or to decomposition. The latter possibility is indicated by an apparent over or under recovery of solute in the first ampoule. Solvents used should be of a high degree of purity and mixed solvents should be avoided if possible; the solvent system chosen should be such that the main component in the sample being examined is the first to saturate the system.